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ROSELI MARIA FORATTO

HARMONIC COMPONENTS FUNCTION IN ACOUSTIC COMMUNICATION OF DENDROPSOPHUS MINUTUS

FUNÇÃO DE COMPONENTES HARMÔNICOS NA COMUNICAÇÃO ACÚSTICA DE DENDROPSOPHUS MINUTUS (PETERS, 1872)

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(2017)

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RESUMO

Nos sistemas de comunicação animal, a relação social entre emissor e o receptor é determinante para modelar as propriedades do sinal acústico. Em anuros a seleção sexual é o principal processo que modifica, dentro da uma escala evolutiva, os cantos de anúncio dos machos de acordo com a preferência e capacidade auditiva das fêmeas em sua população. No entanto, o conhecimento sobre a percepção dos anuros e o comportamento diante das alterações nas diferentes propriedades acústicas dos sinais emitidos em diferentes contextos ainda é incipiente. Assim, nosso trabalho investigou o significado das estruturas harmônicas na comunicação acústica da espécie Neotropical *Dendropsophus minutus*, utilizando dois diferentes experimentos: (1) escolha da fêmea e (2) interação macho-macho. Hipotetizamos que as estruturas harmônicas são necessárias para o reconhecimento específico e escolha do parceiro. Desta forma, esperamos que as modificações nas estruturas harmônicas dos cantos de anúncio provocassem diferentes respostas comportamentais em machos e fêmeas. As fêmeas foram testadas em uma caixa semi-anecóica, onde oferecemos alternadamente por 10 minutos, dois estímulos compostos por notas ABCC (como vocalização da espécie): (1) naturais com harmônicos e (2) sintéticos sem harmônicos. Os experimentos de reprodução com machos ocorreram diretamente em seu sítio de vocalização e testamos três estímulos compostos pela repetição de três notas B apresentados aleatoriamente: (1) natural com harmônicos, (2) sintéticos sem harmônico e (3) editado com o harmônico dominante invertido. Tanto fêmeas quanto machos responderam a todos os estímulos e não discriminaram entre os cantos naturais e sintéticos. Os machos reduziram a frequência dominante das notas A (de anúncio) e aumentaram a taxa de emissão de notas B (agressivas) sobre a influência dos estímulos. Concluímos que a ausência de estrutura harmônica nos sinais acústicos emitidos nos cantos de anúncio de *D. minutus* não prejudica a função de reconhecimento da espécie, não sendo relevante para a seleção sexual. Este trabalho aumenta nosso conhecimento sobre a função de traços acústicos na comunicação dos anuros, e sugerimos que a estrutura harmônica no sistema de comunicação de *D. minutus* é apenas um artefato mecânico neutro da produção de som.

Palavras-chave: Resposta acústica, preferência das fêmeas, canto harmônico, experimento de playback, seleção sexual, discriminação de sinal, territorialidade.

ABSTRACT

In animal communication, the relationship between sender and receiver is determinant for the evolution of signal properties. In anurans, sexual selection is the main process that modifies, within an evolutionary scale, the advertisement calls of males according to the preference and hearing capacity of females. Knowledge on how anurans perceive and react to changes on the structure of acoustic signals is relevant for the classification of the social meaning of particular acoustic properties. We therefore hypothesized that harmonic structures are fundamental for specific recognition and mate choice. Thus, we expected that modifications on harmonic structures of natural calls would provoke different male and female behavioral responses. In order to address that, we investigated the significance of harmonic structures on the acoustic communication of the Neotropical treefrog *Dendropsophus minutus*, in two different playback experiments: (1) female choice, and (2) male-male interactions. Females were tested in a semi-anechoic box, where we offered alternately, for 10 minutes, two stimuli composed by ABCC notes: (1) natural call with harmonics, and (2) synthetic calls without harmonics. Playback experiments with males occurred directly on male's calling site, and we tested three stimuli composed by the repetition of BBB calls in the randomly presented set: (1) natural calls with harmonics, (2) synthetic calls without harmonics, and (3) synthetic calls with the dominant harmonic inverted. Both females and males responded to all stimuli and were unable to discriminate among natural and synthetic calls. Males usually reduced dominant frequency of notes A (advertisement call) and increased the rate of B (aggressive call) notes, under the influence of stimuli. We conclude that the absence of harmonic structure in acoustic signals emitted in the advertising calls of *D. minutus* does not jeopardize the species recognition and is not relevant for sexual selection. This work increases our knowledge on the function of acoustic traits on frog's communication, and we suggest that harmonic structure in the communication system of *D. minutus* is only a neutral mechanic by-product of the sound production.

Keywords: Acoustic responses, female choice, harmonic calls, playback experiment, sexual selection, signal discrimination, territoriality

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Introdução geral

A comunicação acústica é um importante componente do comportamento dos anuros (Bodnar 1996). Muitos animais são capazes de produzir e perceber sons com alta complexidade em sua comunicação (Simmons 1988; Simmons et al. 2003; Vielliard 2004), entre eles há os que apresentam a capacidade de produzir sons harmônicos, que consistem em vibrações sonoras originadas em diferentes regiões do órgão vibrante (Köhler et al. 2017), com a presença de uma frequência fundamental (primeira faixa de frequência), seguida por faixas complementares que são múltiplos inteiros da frequência fundamental (Schwartz and Simmons 1990; Blackstock 2000; Vielliard and Silva 2008; Toledo et al. 2015).

Em invertebrados temos o exemplo da ordem Orthoptera, em que a presença do harmônico em sua comunicação acústica influenciou nas relações de predação, seleção sexual e competição interespecífica por nicho (Robillard et al. 2007). Já em vertebrados os estudos indicam que o harmônico está relacionado a comunicação realizada a longa distância por indivíduos da mesma espécie, como no primata *Galago senegalensis senegalensis* (Zimmerman 1985), em Elefantes, Rinocerontes e Leões (O'Connell-Rodwell et al. 2001).

A comunicação acústica exerce um papel fundamental nas questões relativas à sobrevivência e reprodução dos animais (Ryan 1991; Vielliard 2004). Isso porque tais sinais acústicos produzidos por muitos vertebrados e invertebrados podem auxiliar no reconhecimento específico (Wells 2007; Duellman 1970) e até em sistemas de defesa (Charnov and Krebs 1975; Sherman 1977; Toledo et al. 2015). A complexidade vocal muitas vezes é fruto da evolução de processos de divergência específica e de forças exercidas pela pressão de seleção sexual (Amézquita et al. 2005), entretanto também podem ser fruto consequências da evolução biomecânica (Akre et al. 2014). A pressão exercida pelas fêmeas sobre as características acústicas apresentadas pelos machos de sua espécie pode ser muito significativa (Bee et al. 2000a; Gerhard and Huber 2002). Pois a escolha realizada pelas fêmeas avalia um conjunto de informações presentes no canto como intensidade do canto, taxa de notas e duração do canto (Wells and Schwartz 2007), sendo esses parâmetros fundamentais também para o estudo da comunicação acústica (Fine and Parmentier 2015). Tais informações são condicionadas a características morfológicas e fisiológicas do emissor (Boeckle et al. 2009), dessa forma, as propriedades acústicas do som emitido dependem do tamanho do animal (Boeckle et al. 2009; Kelley 2001), do formato e das características do órgão vocal (Boeckle et al. 2009; Kelley

2001). Os anuros vocalizam por meio de contrações musculares da parede do corpo do animal, forçando o ar contido nos pulmões a passar da laringe para o saco vocal (Narins and Feng 2007). O saco vocal atua apenas como um órgão ressonante, porém as características da laringe, desde estrutura à tensão das cordas vocais, podem interferir significativamente na qualidade do som produzido (Bositel et al. 2011; Narins and Feng 2007).

Em geral, entre os anuros o som é primeiramente captado pelo tímpano (orelha externa), transportado pelo estribo (orelha média) até a orelha interna, onde é traduzido em sinais elétricos, que apresenta dois órgãos sensoriais responsáveis pela recepção do som: a papilla amphibiorum (PA) e a papila basilar (PB) (Narins and Feng 2007). Algumas características do sistema auditivo dos anuros são compartilhadas pelos diferentes grupos de vertebrados (Simmons 1988), e por isso é esperado que espécies não humanas de vertebrados também apresentem a capacidade de compreensão harmoniosa. Isso foi constatado por estudos realizados com *Hyla cinerea* apontam que a espécie é capaz de distinguir sons harmônicos, o que sugere a possibilidade de que demais espécies de anuros possuam tal capacidade (Gerhardt et al.1990) No entanto, a efetividade de atração de fêmeas por sinal de anúncio com harmônicos ainda precisa ser avaliada em outras espécies, assim como a importância deles na comunicação entre machos. Assim, o presente trabalho testou a efetividade de componentes harmônicos nas vias de comunicação macho-fêmea e macho-macho.

Introduction

Acoustic signals produced by animals are crucial for intraspecific communication, which may affect territorial defense, spatial location and reproduction (Goodenough et al. 1993, Breed and Moore 2012). Such signals are acoustically characterized at least by the variation on three dimensions: frequency, time, and amplitude (Snowdon 2011; Wilkins et al. 2012). In anuran advertisement calls, acoustic traits in these dimensions usually have social functions, which may be linked to species recognition (Kollarits et al. 2017), mate choice (Bush et al. 2002), and territorial conflicts (Davies and Halliday 1978). For example, most anurans have hearing structures tuned to the dominant frequency of the advertisement call of their own species (Fuzessery and Feng 1981, Gerhardt and Schwartz 2001; Phelps 2007). Therefore, sound frequencies produced are key to species proper communication. At the same time, motivational state, body size and vocal sac morphology may determine some of these acoustic properties in advertisement calls (Kelley 2001, Boeckle et al. 2009). For example, we expect that larger males normally call with lower frequencies (Gingras et al. 2013), while those sexually more excited call with higher rates than those less excited (Klump and Gerhardt 1987). Therefore, variations in call parameters are frequent and may influence in intraspecific communication.

Evaluation of the impact of such variations on behavioral responses of individuals constitutes an important way for determining their social role. Many studies found that females usually choose the nearest, larger and more motivated males to reproduce, based on signal amplitude, frequency, and advertisement duration call (Klump and Gerhardt 1992; Tárano and Herrera 2003; Beckers and Schul 2004; Márquez et al. 2008). The harmonic structure is composed of frequency bands complementary to a fundamental frequency band (first band), with the complementary bands being multiples of the fundamental frequency (Watkins 1968; Schwartz and Simmons 1990; Vielliard and Silva 2008; Toledo et al. 2015 Fig. 1), originated from sound vibrations in different regions of the vibrating organ (Köhler et al. 2017). These effects are relatively well known for many call traits in anurans; however, the possible functions of the harmonic structure haven’t received much attention in the current literature. For individuals living in noisy environments the harmonic has a function of overlapping the corner with ambient noise, as seen by members of the Hylodidae family (Vielliard and Cardoso 1996; Haddad and Giaretta 1999; Lingnau and Bastos 2007; Pimenta et al.2008; Caldart et al. 2011; Caldart et al. 2016). Studies with *Hyla cinerea* indicate that the species is able to distinguish harmonic sounds, which suggests the possibility that other species of anurans have such a capacity (Gerhardt et al.1990)

With the exception of deaf frogs (e.g., Goutte et al. 2017), anurans generally have a broad spectral sensitivity, which may even include ultrasonic frequency bands (Feng et al. 2006). Also, the registration of the corners can depend on the amount of selectivity of the neurons as characteristics of the singing (Adler and Rose 1998; Edwards et al. 2002; Gerhardt and Bee 2007). Typical tuning curves in anurans respect a bimodal pattern, with, at least, two best excitatory frequencies, generally matching with fundamental and dominant frequency of the species call (Gerhardt and Schwartz 2001). Therefore, we may expect that higher frequencies, received mostly by the basilar papila (Wilczynski and Capranica 1984), are enabled to be heard and perform some function or make the signal more attractive. However, previous playback experiments showed contrasting results by not finding a pattern of preference for harmonic signals considering responses to stimuli with frequency ratios of harmonically structured calls (see Gehardt 1974; Akre et al. 2014).

Here we hypothesized that harmonic structures of the advertisement call from the Neotropical treefrog *Dendropsophus minutus* are crucial for specific recognition and female preference. Thus, we tested such prediction in playback experiments, exposing females and males to natural and synthetic calls (without harmonics or with the dominant harmonic inverted).

Methods

Model species

The Neotropical treefrog Dendropsophus minutus is a well-known species regarding naturalistic aspects (Cardoso and Haddad 1984; Haddad and Cardoso 1992; Morais et al. 2012; Toledo et al. 2015). It's described from the state of Rio de Janeiro, southeastern Brazil, but has a large distribution occurring in different countries from South America (Frost 2017). However, many different lineages were recognized, what possibly represents a cryptic species complex (Hawkins et al. 2007; Gehara et al. 2014). The species is common and is a perfect model to our study, because males present a complex call, usually composed by three types of harmonic notes, A, B and C (Cardoso and Haddad 1984; Toledo et al. 2015). Most energy is usually concentrated in the second harmonic (Cardoso and Haddad 1984). However, these notes are different regarding duration, dominant frequency, and pulses' structure. Notes A and C are similar, pulsed (Cardoso and Haddad 1984), and have the function of mate attraction (Haddad and Cardoso 1992; Toledo et al. 2015 Fig. 1). The advertisement call is usually composed of the sequence of ABCC notes (Cardoso and Haddad 1984; Toledo et al. 2015 Fig. 2). Note B is not pulsed, usually lower, and has a social function related to territoriality and aggressiveness between males (Cardoso and Haddad 1984; Haddad and Cardoso 1992; Toledo et al. 2015 Fig. 1).

Sites

We run playback field experiments with three populations of *D. minutus* from the state of São Paulo, southeastern Brazil, in the following municipalities: (1) Sorocaba (between 15 and 20 November 2015); (2) Ribeirão Grande (between 13 and 15 January 2016); and (3) São Luiz do Paraitinga (at 29 December 2015). Geographic coordinates, climate and vegetation classification are present in the Table S1.

Recordings, preparation and analyses

For each population we prepared stimuli for two playback experiments: female choice and male-male interaction. Before editing the audios, in the first day of fieldwork in each locality we recorded a series of calling males: 7 from Sorocaba, 3 from Ribeirão Grande, and 3 from São Luiz do Paraitinga. We used a microphone Sennheiser ME67 coupled to a Zoom H4N digital recorder for recording a sequence of calls with males about 1 m from the microphones. Recordings were obtained with 24 bits of resolution and 96 kHz of sampling rate. These calls were immediately analyzed in the software Raven Pro 1.4 (Bioacoustics Research Program 2011 – Cornell Lab of Ornithology). From these recordings, we measured call rate, note duration, and dominant frequency. Note duration was obtained in the oscillograms, and we used FFT (Fast Fourier Transformation) 1024 points, and 50% of window overlap to obtain values of dominant frequency. Based on our sample we selected the male's calls that were closest to the average on each population for edition and usage in the experiments. For calibrating the signal intensity in the experiments for each site, we measured the sound pressure level of 4 males, calling at 0.5 m using a SPL meter (Instrutherm DEC-490 model) with the following settings: range of 30 to 130 dBC, and fast time weighting.

For edition, using the software Raven, we used bandpass filter to decrease background noise and export specific notes to the playback audio files. In the female choice experiments, for both stimuli, we edited a sequence of ABCC notes as a call unit repeated during 10 minutes with a call rate of 9 calls/minute. Each call had about 1 s of duration and the same silence interval of 5 s. The treatment-audio file was prepared without harmonic structures using the bandpass filter tools in Raven (Fig. 1). In the male-male interaction experiment we edited a sequence of BBB notes as a call unit (aggressive signal), repeated during 1 minute, with a call rate of 23 calls/minute. Each call had about 1 s of duration and the same silence interval of 1.6 s. This experiment had three different stimuli: (1) natural calls; (2) synthetic calls without harmonics (only the dominant frequency remained); and (3) synthetic calls with the dominant harmonic inverted. In the condition (3) we replaced the energy power equivalently from harmonic 2 to the harmonic 1. Therefore, the dominant frequency was lowered (Fig. 2). For this last edition we used the tool "spectrum filter" in the software Goldwave v6.19. The final edition of all stimuli used in both experiments passed by note normalization at -0.8 dB with Audacity 2.1.1 software.

Female choice experiment

We tested a total of 28 females from São Luiz do Paraitinga, which were captured in the breeding site and conditioned in plastic bags moistened until the experiment, which occurred between 9:40 p.m. and 4:15 a.m. on the same day, and was conducted outdoor near the installations of the accommodation-house at the field station (about 150 m from the breeding site), what favored a minimization of the stress caused by the experiment. After the capture, we submitted each female (once) to the acoustic stimuli (natural and synthetic calls without harmonics), offered alternately from two different 25 W (RMS) speakers (JBL-GT 328, kHz), inside a semi-anechoic arena with the following dimensions: 1 x 0.45 x 0.64 m (Fig. 1). Both speakers were attached to an amplifier (Taramps TS- 150X2, 2 Ohms, maximum power of 150 Watt RMS and frequency range of 18 Hz to 40 kHz), which was connected to a Sony Vaio laptop for experiment controlling. Sound pressure level for the stimuli was equalized to 97.4 and 95 dBC at 0.5 m in São Luiz do Paraitinga and Ribeirão Grande, respectively. The arena had attenuation foam on the walls for background noise reduction and to improve the propagation quality of acoustic stimuli (Fig. S1). We positioned the speakers side by side (30 cm from each other) at one ending of the rectangular arena, and, in each experiment, we released the female on the pavement in the other end, 70 cm from the speakers. The specific disposition of tested females and equipment can be visualized in Fig. 2. All experiments were recorded with a Sony DCR –SR47 camera (videos were deposited in the Fonoteca Neotropical Jacques Vielliard following the access numbers: FNJV 1000538-79), and illuminated with red light 150 W lamp placed about 2 m from the arena, at a height sufficient for complete illumination of the arena floor. We achieved positive phonotaxis with 25 females attracted for at least one of the two stimuli. We delimitated 4 rectangular sections on the arena floor with yellow adhesive tape to obtain behavioral measurements. The sections II and III measured 40 x 22 cm and represented the area for the speakers' selection. The adjacent sections I and VI measured 60 x 22 cm and represented neutral area for the choice experiment (Fig. 2). We quantified three variables: choice prevalence (%), interest timing (s), and latency (s).

We counted stimulus chosen when the female passed for three stages: i) directed the head toward one of the speakers, ii) walked or jumped towards one of them, and iii) positioned, firstly, within the II or III sections indicated on the arena floor. This sequence indicates the first and original choice, counted as official, even if the female approached the other speaker after an unsuccessful search for the "first male". We measured 'interest timing' by the time interval spent into the speaker area (once many females approached both speakers), and "latency" as the time interval between the stimulus and the first female head tracking.

Male-male interaction experiment

We run playback experiments with a total of 13 males (7 from Sorocaba, 3 from São Luiz do Paraitinga and 3 from Ribeirão Grande), which were found vocally active in breeding sites, between 19:52pm and 00:24am. Males selected for the experiment were calling, however, with distances larger than 1 m from the nearest neighbor. After we found a focal-male, we placed the equipment to reproduce the stimuli and for recording the behavioral and acoustic data. The speaker was placed about 50 cm from the focal individual to simulate an invader male (Fig. 3). We used an Instrutherm TR700 laser distance measurer for obtaining such distances. For each locality we adjusted the speaker sound pressure level (mean based in previous measurements) for the stimuli using a SPL meter 0.5 m from the speaker inside a semi-anechoic box. For males from Sorocaba we used 84.9 dBC, 95 dBC for males from Ribeirão Grande, and 94.8 dBC in São Luiz do Paraitinga. We used the same speaker model, amplifier, red lamp (about 2 m from focal males) and computer to run, illuminate and control the playback as was described in the experiment for female choice. For recording acoustic responses, we used a Zoom H4N digital recorder with a directional microphone Sennheiser ME67 supported by a tripod about 1 m from the focal male.

For each male, we tested, sequentially, three stimuli (natural with harmonics, synthetic without harmonics, and synthetic with dominant harmonic inverted) with randomized order and interval, at least longer than 13 min, to achieve independence among acoustic treatments. However, each phase of the experiment only started when males were vocally active. For each acoustic treatment we made recordings and observations on the focal-male in three experiment stages: i) two min before (pre-stimulus), ii) one min during (simultaneous), and iii) two min after (post-stimulus) the stimulus. In order to capture the effect of the acoustic stimuli, we evaluated on each male, in all stages of the experiment (pre, simultaneous, and post-stimulus), the rate of call emission, dominant frequency, duration of notes A, B, and C, and if there was movement towards the signal. Males were captured and kept in plastic bags after the sequence of acoustic treatments, for body size (snout-vent length: SVL) measurements, using a digital caliper rule Mitutoyo with accuracy 0.1 mm. This procedure avoided males participating more than one time in the experiments. We released all males at the end of the experiments in each breeding site. Data on air temperature, humidity, hour during each experiment, as well as, males' SVL are in Table S2.

Data analysis

We used a G test for evaluating statistic difference in females' choice prevalence among natural and synthetic (without harmonics) calls. Interest timing and latency among acoustic stimuli were compared through paired *t* test, once we found normal distribution. To access if males responded differently to natural and synthetic calls (without harmonics and with dominant harmonic inverted), we applied a sequence of ANOVAs comparing rate, dominant frequency, and duration of notes A, B, and C among different experiment stages. The *Post-hoc* test of Fischer was used for finding specific differences of acoustic properties among experiment stages for each stimulus. We performed the statistical analysis in the software Bioestat 3.0 (Ayres et al. 2003) and Statistica 7.1 (StatSoft 2005).

Results

Female choice

Most females (80%) choose the synthetic (without harmonics) over the natural call (G = 3.986, Log-likelihood = 2; $P = 0.045$; n = 25) (Fig. 4A). We detected difference in interest timing (t = 0.6.721; $P < 0.001$; n = 25) and difference in latency ($t = 1.296$; $P = 0.27$; n = 25) among stimuli (Fig. 4B and C).

Male-male interaction

Most males (53.3 %) reacted aggressively, moving the head towards the sound source and adopting the body raising posture, independent of the stimuli used in the playback. Males usually modified their calls in response to all types of stimuli (Fig. 5). However, each stimulus produced specific responses with significant differences. The rate of emission of note C, presents a significant difference for the three treatments applied. The treatment with synthetic with dominant harmonic inverted is P = 0.032 and P = 0.024, between Pre-stimulus and Simultaneous, and between Simultaneous and Post-stimulus respectively. The synthetic without harmonic test showed a difference between Pre-stimulus and simultaneous P = 0.01 and simultaneous and post-stimulus P = 0.024, following the same difference pattern between treatment. The test with the Natural with harmonic, presents a difference between pre-stimulus and simultaneous P = 0.015 and simultaneous and post-stimulus P = 0.03 (Table 1).

Although there were no significant results, other differences could be observed during the Playback with natural calls, which provoked changes on all notes rate of emission and dominant frequency of note A. Synthetic calls without harmonics provoked modifications on rate of notes B and C, and dominant frequency of note A; while the treatment with dominant harmonic inverted provoked modifications on rate of emission of note B, and dominant frequency and duration of note A. Patterns of such modifications were similar among different stimuli. Rate of emission of notes A were normally reduced with the stimuli, while rate of emission of notes B and C increased, mainly in the simultaneous experimental stage. Dominant frequency and duration of note A always decreased as response to the stimuli (Table 1; Fig. 5).

Discussion

Different of what we expected, once individuals from both sexes emitted response to all stimuli tested, the absence of harmonic structures on the advertisement call of the Neotropical treefrog D. minutus did not affect the species recognition and the intraspecific communication system. Apparently, both females and males recognized the intraspecific signals even with modifications on the harmonic structure (Ryan et al. 1992; Ryan and Rand 2001; Akre 2014). This shows that the context of species recognition on acoustic signals, exposes focal individuals to strange signals and conspecific calls, finding strong positive phonotaxis to conspecific calls. In this context, we understand that our synthetic modifications on harmonic structures of natural calls were not sufficient to avoid the species recognition. Advertisement calls carry many properties that ensure the species recognition (Gerhardt 1982). However, there is a consensus that dominant frequency is the main acoustic property related to species recognition, and, it is likely that we found positive behavioral responses, because we kept all original acoustic properties and, at least, a minimum energy on the regular band of dominant frequency of the species, in the synthetic signals tested, since D. minutus naturally inverts the energy of the harmonics (LFT, personal observation).

Although females apparently preferred synthetic (without harmonics) over natural calls, we found differences on interest timing and not in latency among stimuli, which supports the hypothesis that females are not able to discriminate among the tested signals. Thus, both signals were equally attractive for them. This result matches with what was previously found for the túngara frog (Physalaemus pustulosus), where females were indifferent to harmonic calls (Akre et al. 2014). Females' choice is influenced by multiple acoustic factors (Gerhardt and Brooks 2009), but possibly not for harmonic structures. Such results can suggest the hypothesis that harmonic calls did not evolve as a response to females' preference on sexual selection, maybe characterizing the harmonic structures as a neutral feature and a mechanic by-product of the sound production.

In opposition to our findings, Gerhardt (1974) tested frequency preference by phonotaxis in females of the hylid Dryophytes cinereus, and noted that there was maximized response to acoustic stimulus having a bimodal spectrum. Females were more responsive according to a specific energy distribution on frequency bands (Gerhardt 1974). It is relatively well accepted that female's auditory systems, by the spectral sensitivity, determines such preferences. Many species of frogs have hearing abilities with best excitatory frequencies in a bimodal pattern (Gerhardt and Schwartz 2001), possibly matching with fundamental and dominant frequency of their own species call. Anecdotally, Rose et al. (1988) observed females' response to playback of calls characterized only by the fundamental frequency.

In some cases, harmonic calls are able to stimulate, at the same time, both sensitive organs in the inner ear, the amphibian and basilar papillae (Ryan and Rand 1990). As the sensory exploitation theory predicts, harmonic calls should increase signal attractiveness, making our results contradictory. Therefore, we indicate the need for additional playback experiments with D. minutus and other species, coupled with physiological essays to understand the spectral sensitivity and best excitatory call frequencies. These trials would give us a general idea of the significance of harmonic calls for the male-female communication in anurans.

It is important to note that males and females may present substantial differences on auditory tuning and response to different call frequencies, mainly when the species has complex calls (Narins and Capranica 1976). Different notes in the complex advertisement call of D. minutus probably act with different social roles, according to the evidences we have (Haddad and Cardoso 1992; Toledo et al. 2015). Notes A and B have different dominant frequencies, and while note A (higher dominant frequency) is determinant for mate attraction (directed for females), the note B (lower dominant frequency) is fundamental to solve territorial conflicts (directed for males) (Toledo et al. 2015). There may be no relevant differences in the best excitatory frequency between the sexes of D. minutus, since both males and females responded to synthetic calls, and differences of dominant frequency among notes are not so strong.

Males were aggressive and changed their calls in response to the three stimuli tested. Although, some stimuli did not produce significant differences in acoustic responses, the patterns of males' modifications on acoustic signals after the stimuli were similar (Fig. 4). Generally, males reduced the dominant frequency of notes A and C and increased the rate of the aggressive notes (B) for any kind of competitor simulated. Similar changes on dominant frequency and aggressive calls rate are common responses among territorial males in anurans (Wells 1988; Wagner 1989; Bee et al. 2000b; Bee and Bowling 2002; Gardner and Graves 2005; Nali and Prado 2014; Fang et al. 2014; Morais et al. 2015).

The substantial reduction on the duration of notes A after the stimuli, suggests that this note have a minor importance during territorial encounters and supports the hypothesis that their social role is related to mate attraction, once males reduce the dominant frequency in the presence of an imminent competitor (Haddad and Cardoso 1992). Regarding the negative relationship between call frequency and body size (Gingras et al. 2013) we would expect that the stimulus composed by dominant harmonic inverted (simulating a larger male) would provoke different responses in territorial males than that observed with other stimuli. But, it is possible that territorial individuals of D. minutus use the same pattern of aggressive response to invaders, independently of spectral properties (adopting the bourgeois strategy, where the territorial male faces the invader independent of the characteristics of the invading male, see: Maynard Smith and Parker 1976), as a mechanism to neutralize dishonest males (Bee et al. 2000b).

This work increases our knowledge on the functions of acoustic traits during the intraspecific communication among anurans. Herein we conclude that the harmonic structure present in the advertisement call of D. minutus have no special role on species recognition and on sexual selection, once both males and females were not able to discriminate among synthetic and natural calls.

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Considerações Finais

A partir das análises realizadas compreendemos que o componente harmônico não oferece informações essenciais para o reconhecimento específico de *D. minutus*, seja na comunicação macho-macho ou macho-fêmea, contrariando a perspectiva que inicialmente tínhamos. Entretanto, todas as modificações realizadas nos cantos e emitidas nos *playbacks* puderam ser reconhecidas pelos indivíduos testados, demonstrando que o reconhecimento específico independe do harmônico no qual a frequência dominante está inserida. Tais fatos nos levam a sugerir que a estrutura harmônica presente no canto de diversas espécies de anuros, possivelmente independente de uma pressão seletiva gerada pela escolha das fêmeas. Em outros grupos como em Elefantes e Rinocerontes sabe-se que o harmônico detém a importante função de favorecer a propagação da informação a longas distâncias, o que nos leva a indagar se para os anuros o harmônico também pode possuir alguma função relacionada à comunicação a longa distância.

Assim, considerando as diversas formas de comunicação existentes, há necessidade de que sejam conduzidos novos estudos sobre o tema, abrangendo a comunicação acústica macho-macho e macho-fêmea em anuros, que apresentem tanto hábito quanto sítio de reprodução diferentes de *D. minutus* para que assim possamos compreender as possíveis variações do significado biológico do harmônico para a comunicação acústica.

References

Adler TB, Rose GJ (1998) Long-term temporal integration in the anuran auditory system.Nat Neurosci 1:519-523.

Akre KL, Ximen AB, Rand AS, Ryan MJ (2014) Harmonic calls and indifferent females: no preference for human consonance in anuran. Proc. R. Soc. 281: 20140986. doi: 10.1098/rspb.2014.0986.

Amézquita A, Castellanos L, Hödl W (2005) Auditory matching of male *Epipedobates femalis* (Anura: Dendrobatidae) under Field conditions. Animal Behaviour, v. 70, p. 1377-1386.

Ayres M, Ayres Junior M, Ayres DL, Santos AS (2003) Sociedade Civil Mariraúá/MCT-CNPq. Belém, PA, Brasil.

Baider C, Tabarelli M, Mantovani W (1999) O Banco de sementes de um trecho de Floresta Atlântica Montana (São Paulo, Brasil). Rev Brasil Biol 59(2):319-328.

Balbani APS, Montovani JC (2008) Aspectos filogenéticos da audição. Rev Bras Med 2:37-45.

Bastos RP, Haddad CFB (1996) Breeding activity of the Neotropical treefrog *Hyla elegans* (Anura, Hylidae). J Herpetol 30(3):355-360.

Beckers OM, Shul J (2004) Phonotaxis in *Hyla versicolor* (Anura, Hylidae): the effect of absolute call amplitude. J Comp Physiol A 190(11):869-876.

Bee MA, Bowling AC (2002) Socially mediated pitch alteration by territorial male bullfrogs, *Rana catesbeiana*. J Herpetol 36(1):140-143.

Bee MA, Perrill SA, Owen PC (2000) Male green frogs lower the pitch of acoustic signals in defense of territories: a possible dishonest signal of size? Behav. Ecol (2):169–-177. doi: 10.1093/beheco/11.2.169

Bee MA, Kozich CE, Blackwell KJ, Gerhardt HC (2000) Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: implications for individual discrimination. Ethol 107:65-84. doi: 10.1046/j.1439-0310.2001.00640.

Blackstock DT (2000) Fundamentals of physical acoustics. 1st ed. New York: John Willey and Sons Inc.

Boeckle M, Preininger D, Hödl W (2009) Communication in noisy environments I: Acoustic signals of *Staurois latopalmatus* Boulenger, 1887. Herpetologica 65 (2):154-165. doi: doi.org/10.1655/07-071R1.1

Boistel R, Aubin T, Cloetens P, Langer M, Gillet B, Josset P, Pollet N, Herrel A (2011) Whispering to the Deaf: Communication by a frog without external vocal sac or Tympanum in Noisy Environments. Plos One 6(7):e22080. doi: 10.1371/journal.pone.0022080

Breed MD, Moore J (2012) Animal Behavior Burlington, MA: Academic Press.

Bush SL, Gerhardt HC, Schul J (2012) Pattern recognition and call preferences in treefrogs (Anura: Hylidae) a quantitative analysis using a no-choice paradigm. Anim. Behav (63):7-14. doi:10.1006/anbe.2001.1880

Caldart VM, Iop S, Cechin SZ (2011) Vocalizations of *Crossodactylus schmidti* Gallardo, 1961 (Anura, Hylodidae): advertisement call and aggressive call. North West J Zool 7:118-124

Caldart VM, Iop S, Lingnau R, Cechin SZ (2016) Communication in a noisy environment: short-term acoustic adjustments and the underlying acoustic niche of a Neotropical stream-breading frog. Acta Ethol 19:151-162. doi: 10.1007/s10211-016-0235-2

Capranica RR (1965) The evoked vocal response of the Bullfrog: A study of communication by sound. Cambridge: MIT.

Cardoso AJ, Haddad CFB (1984) Variabilidade acústica em diferentes populações e interações agressivas de *Hyla minuta* (Amphibia, Anura). Ciênc Cult 36(8):1393-1399.

Cepagri (centro de pesquisas meteorológicas e climáticas aplicadas a agricultura) (2016). http://www.cepagri.unicamp.br/. Accessed 19 October 2016.

Charnov EL, Krebs J R (1975) The Evolution of Alarm Calls: Altruism or Manipulation? The American Naturalist, v. 109, n. 965, p. 107-112.

Davies NB, Halliday TR (1978) Deep croaks and fighting assessment in toads *Bufo bufo*. Nature 274:683-685.

Duellman WE (1970) The Hylid frogs of Middle America. Monograph of The Museum of Natural History. Lawrence: The University of Kansas.

Edwards CJ, Adler TB, Rose GJ (2002) Auditory midbrain neurons that count. Nat Neurosci 5:934-936.

Fang G, Jiang F, Yang P, Cui J, Braunth SE, Tang Y (2014) Male vocal competition is dynamic and strongly affected by social contexts in music frogs. Anim Cogn 17:483. doi: 10.1007/s10071-013-0680-5

Faria de Oliveira E, Neves Feio R, Pinto da Matta SL (2007) Aspectos reprodutivos de *Dendropsophus minutus* (Peters, 1872) no município de Viçosa, Minas Gerais. Rev Ceres 54(313):230–238.Felsenteins J. (2003) Inferring Phylogenies, Sinauer Associates, 2 ed.

Feng AS, Narins PM, Xu C-H, Lin W-Y, Yu Z-L, Qiu Q, Xu Z-M, Shen J-X. (2006) Ultrasonic communication in frogs. Nature (440):333-336. doi: 10.1038/nature04416

Fine ML, Parmentier E (2015) Mechanisms of fish sound production. In: LADICH, F. (Ed.). Sound Communication in fishes. Vienna: Springer. p. 77-126.

Frishkopf LS, Capranica RR, Goldstein Jr MH (1968) Neural coding in the bullfrog's auditory system: a teleological approach. Proc. I.E.E.E. 56: 969-980.

Frost DR (2017) Amphibian Species of the World: na Online Reference. American Museum of Natural History, New York, USA. Version 6.0. http://www.amnh.org/our-research/vertebratezoology/herpetology/. Accessed 11 May 2017.

Fundação Florestal do Estado de São Paulo. Parque Estadual da Serra do Mar: Núcleo de Santa Vigínia. http://www.parqueestadualserradomar.sp.gov.br/pesm/nucleos/santa-virginia/sobre/ Accessed 19 October 2016.

Fuzessery ZM, Feng AS (1981) Frequency selectivity in the anuran auditory midbrain: Single unit response and multiple tone stimulation. J. Comp Physiol 146:471-484.

Gardner EA, Brent MG (2005) Responses of resident male *Dendrobates pumilio* to territory intruders. J Herpetol 39(2):248-253. doi: 10.1670/95-04A

Gehara M, Crawford AJ, Orrico VGD, et al. (2014) High Levels of Diversity Uncovered in a Widespread Nominal Taxon: Continental Phylogeography of the Neotropical Tree Frog *Dendropsophus minutus.* Plos One 9(9):e103958. doi: 10.1371/journal.pone 0103958.

Gerhardt HC (1974) The significance of some spectral features in mating call recognition in the green treefrog (*Hyla cinerea*). J Exp Biol 61(1):229-41.

Gerhardt HC (1982) Sound Pattern Recognition in Some North American Treefrogs (Anura: Hylidae): Implications for Mate Choice. Integr Comp Biol 22(3):581-595.

Gerhardt HC, Bee MA (2007) Recognition and Localization of Acoustic Signals. In: Narins PM, Feng AS, Fay RR, Popper AN (ed) Hearing and Sound Communication in Amphibians. Springer Handbook of Auditory Research. Springer, New York, NY. doi:10.1007/978-0-387-47796-1_5

Gerhardt HC, Brooks R (2009) Experimental analysis of multivariate female choice in gray treefrogs (*Hyla versicolor*): Evidence for directional and stabilizing selection. Evolution 63-10: 2504-2512. doi: 10.1111/j.1558-5646.2009.00746.x

Gerhardt HC, Schwartz JJ (2001) Auditory tuning and frequency preferences in anurans. Ryan MJ (ed) Anuran communication. Smithsonian Institution Press, Washington, DC, pp 73-85

Gerhardt HC, Huber F (2002) Acoustic Communication in Insects and Frogs: Common Problems and Diverse Solutions. Chicago: University of Chicago Press.

Gerhardt HC, Crawford AJ, Orrico VGD et al (2014) High level of diversity uncovered in a widespread nominal taxon: continental phylogeography of the Neotropical tree frog *Dendropsophus minutus*. Plos One 9(9): e103958. doi: 10.1371/journal.pone.0103958

Gingras B, Boeckle M, Herbst T, Fitch WT (2013) Call acoustics reflect body size across four clades of anurans. J Zool 289:143-150. doi: 10.1111/j.1469-7998.2012.00973.x

Goodenough J, McGuire B, Wallace RA (1993) Perspectives on Animal Behaviour, New York, Wiley.

Goutte S, Mason MJ, Dalsgaard JC, Montealegre-Z F, Chivers BD, Sarria-S FA, Antoniazzi MM, Jared C, Sato LA, Toledo LF (2017) Evidence of auditory insensitivity to vocalization frequencies in two frogs. Sci Rep 7:121. doi: 10.1038/s41598-017-12145-5

Haddad CFB, Giaretta AA (1999) Visual and acoustic communication in the brazilian torrent frog, *Hylodes asper* (Anura: Leptodactylidae). Herpetologica 55:324-333.

Haddad CFB, Cardoso AJ (1992) Elección del macho por la hembra de *Hyla minuta* (Amphibia: Anura). Acta Zool Lilioana 41:81-91.

Hawkins MA, Sites Jr, JW, Noonan, BP (2007) *Dendropsophus minutus* (Anura: Hylidae) of the Guiana Shield: using DNA barcodes to assess identity and diversity. Zootaxa. 5140:61-67.

Heap S, Stuart-Fox D, Byrne P (2011) Variation in the effect of repeated intrusions on calling behavior in a territorial toadlet. Behav Ecol 23(1):93-100. doi: 10.1093/beheco/arr161

Heyer WR, Rand AS, Cruz CAG, Peixoto O, Nelson CE (1990) Frogs of Boracéia. Arq Zool 31:231-410.

IBGE, Departamento de Recursos Naturais e Estudos Ambientais (1992). Manual Técnico da Vegetação Brasileira. Série manuais técnicos em geociências, 1. Rio de Janeiro.

Kelley DB, Tobias ML, Horng S (2001). In: Ryan MJ (ed) Anuran Communication. Smithsonian Institution Press, Michigan.

Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel MO, Vences M (2017) The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. 4251(1):001-124. doi:10.11646/zootaxa.4251.1.1

Kollarits D, Wappl C, Ringler M. (2017) The role of temporal call structure in species recognition of male *Allobates talamancae* (Cope, 1875) (Anura: Dendrobatidae). Herpetozoa (29):115-124.

Klump GM, Gerhardt HC (1987) Use of non-arbitrary acoustic criteria in mate choice by female gray tree frog. Nature 326:286-288.

Klump GM, Gerhardt HC (1992) Mechanisms and function of call-timing in male-male interactions in frogs. In: McGregor PC (ed) Playback and studies of animal communication. Plenum Press, New York, 153-174.

Lingnau R, Bastos RP (2007) Vocalizations of the Brazilian torrent frog *Hylodes heyeri* (Anura: Hylodidae): repertoire and influence of air temperature on advertisement call variation. J Nat Hist 41:1227-1235. doi:10.1080/00222930701395626

Littlejohn MJ, Loftus-Hills JJ. (1968) An experimental evaluation of pre-mating isolation in the *Hyla ewingi* complex (Anura: Hylidae). Evol 22:659-663.

Márquez R, Bosch J, Eekhout X (2008) Intensity of female preference quantified through playback setpoints: call frequency versus call rate in midwife toads. Anim. Behav 75:159-166. doi: 10.1016/j.anbehav.2007.05.003

Martof BS, Thompson BM (1970) Reproductive behaviour of the chorus frog *Pseudacris nigrita*. Behav 13:243-248.

Maynard Smith J, Parker GA (1976) The logic of asymmetric contests. Anim Behav 24:159-177.

Morais AR, Batista VG, Gambale PG, Signorelli L, Bastos RP (2012) Acoustic communication in a Neotropical frog (*Dendropsophus minutus*): vocal repertoire, variability and individual discrimination. Herpetol J 22:249-257.

Morais AR, Siqueira MN, Bastos RP (2015) How do males of *Hypsiboas goianus* (Hylidae: Anura) respond to conspecific acoustic stimuli? Zoologia 32(6):431-437. doi: 10.1590/s1984-46702015000600001

Nali RC, Prado CPA (2014) Complex call with different messages in *Bokermannohyla ibitiguara* (Anura, Hylidae), a gladiator frog of the Brazilian Cerrado. J Herpetol 48(3):407-414. doi: 10.1670/13-090

Narins P, Feng A (2007) Hearing and Sound Communication in Amphibians. Ed. Springer, pp 1-11. doi.org/10.1007/978-0-387-47796-1_1

Narins PM, Capranica RR (1978) Commucative significance of the two-note call of the treefrog *Eleutherodactylus coqui*. J Comp Physiol 127:1-9.

Narins PM, Capranica RR (1976). Sexual differences in the auditory system of the tree frog *Eleutherodactylus coqui*. Science 192(423):378-380. doi: 10.1126/science.1257772

Narvaes P, Rodrigues MT (2005) Visual communication, reproductive behavior, and home range of *Hylodes dactylocinus*: (Anura, Leptodactylidae). *Phyllomedusa* 4:147-158. doi: 10.11606/issn.2316-9079.v4i2p147-158

O'Connell-Rodwell CE, Hart LA, Arnason BT (2001) Exploring the potential use of seismic waves as a communication channel by elephants and other large mammals. American Zoologist, v. 41, n. 5, p. 1157-1170.

Pimenta BVS, Wachlevski M, Cruz CAG (2008) Morphological and acoustical variation, geographic distribution, and conservation status of the spinythumb frog *Crossodactylus bokermanni* Caramaschi and Sazima, 1985 (Anura, Hylodidae). J Herpetol 42:481-492.

Phelps SM (2007) Sensory ecology and perceptual allocation: new prospects for neural networks. Phill Trans R Soc B 362:355-367. doi: 10.1098/rstb.2006.1963

Rand AS, Ryan MJ, Wilczynski W (1992) Signal redundancy and receiver permissiveness in acoustic mate recognition by the tu´ngara frog, *Physalaemus pustulosus*. Am. Zool. 32, 81-90.

Robillard T., Desutter-Grandcolas L (2006) Phylogeny of the cricket subfamily Eneopterinae (*Orthoptera, Grylloidea, Eneopteridae*) based on four molecular loci and morphology. Molecular Phylogenetics and Evolution, v. 40, p. 643-661.

Ron SR (2008) The evolution of female mate choice for complex calls in túngara frogs. Anim Behav 76:1783-1794. doi: 10.1016/j.anbe.hav.2008.07.024

Rose GJ, Zelick R, Rand AM (1988) Auditory Processing of Temporal Information in a Neotropical Frog is Independent of Signal Intensity Ethol 77:330–336. doi: 10.1111/j.1439-0310.1988.tb00214.x

Ryan MJ (1985) The Túngara Frog, A Study in Sexual Selection and Communication, University of Chicago Press, Chicago.

Ryan MJ (1988) Constraints and patterns in the evolution of anuran acoustic communication. The evolution of the amphibian auditory system, pp 637-677.

Ryan MJ (1991) Sexual selection and communication in frogs. Trends Ecol Evol 6(11):351-355.

Ryan MJ, Keddy-Hector A (1992) Directional patterns of female mate choice and the role of sensory biases. Am Nat 139 (suppl.):S4-S35.

Ryan MJ, Rand A (1990) The sensory basis of sexual selection for complex calls in the túngara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). Evol 44:305-314. doi: 10.1111/j.1558-5646.1990.tb05200.x

Ryan MJ, Rand AS (2001) Feature weighting in signal recognition and discrimination by túngara frogs. In: Ryan MJ (ed) Anuran communication. Smithsonian Institution, Washington. DC pp 86-101.

Schartz JJ. Simmons AM (1990) Encoding of a spectrally-complex communication sound in the bullfrog's auditory nerve. J Comp Physiol A 166: 489-499.

Shen JX, Xu ZM, Yu ZL, Wang S, Zheng DZ, Fan SC (2011) Ultrasonic frogs show extraordinary sex differences in auditory frequency sensitivity. Nat Com 2:342. doi: 10.1038/ncomms1339.

Sherman PW (1977) Nepotism and the Evolution of Alarm Calls. Science, v. 197, n. 4310, p. 1246-1253.

Simmon AM (1988) Selectivity for harmonic structure in complex sounds by the green frog (Hyla cinerea). Journal of Comparative Physiology A, v. 62, p. 397-403.

Shofner WP, Feng AS (1981) Post-metamorphic development of the frequency selectivities and sensitivities of the peripheral auditory system of the bullfrog *Rana catesbeiana*. J Exp Biol 93:181-196.

Starnberger I, Preininger D, Hödl W (2014) From uni-to multimodality: towards an integrative view on anuran communication. J Comp Physiol A. 200:777-787. doi: doi 10.1007/s00359-014-0923-1

Statsoft Inc (2005) STATISTICA (data analysis software system), version 7.1. www.statsoft.com.

Toledo LF, Martins IA, Bruschi DP, Passos MA, Alexandre C, Haddad CFB (2015) The anuran calling repertoire in the light. Acta Ethologica, v. 18, n. 2, p. 87-99.

Tárano Z, Herrera EA (2003) Female Preferences for Call Traits and Male Mating Success in the Neotropical Frog *Physalaemus enesefae.* Ethol 109:121-134. doi: 10.1046/j.1439-0310.2003.00848.x

Toledo LF, Martins IA, Bruschi DP, Passos MA, Alexandre C, Haddad CFB (2015) The anuran calling repertoire in the light. Acta Ethol 18(2):87-99. doi: 10.1007/s10211-014-0194-4

Vélez A, Höbel G, Gordon NM, Bee MA (2012) Dip listening or modulations masking? Call recognition by green treefrogs (*Hyla cinerea*) in temporally fluctuating noise, J Comp Physiol A 198:891904. doi: 10.1007/s00359-012-0760-z.

Vielliard JMA (2004) Diversidade de Sinais e Sistemas de Comunicação Sonora na Fauna Brasileira. In: Seminário Música Ciência Tecnologia: Acústica Musical, Universidade de São Paulo, Instituto de Matemática e Estatística, 1:145-152.

Vielliard JME, Cardoso AJ (1996) Adaptação de sinais sonoros de anfíbios e aves a ambientes de riachos com corredeiras. In Pefaur JE (ed) Herpetologia neotropical. Acta Del II Congr. Lat. Am. Herpetol. Univ. Los Andes. Consejo de Desarrolo, Humanistico y Tecnológico, Merida, Venezuela, pp 97-119.

Wagner WE (1989) Fighting, assessment, and frequency alteration in Blanchard’s cricket frog. Behav Ecol Sociobiol 25(6):429-436. doi: 10.1007/BF00300189

Watkins WA (1968) The harmonic interval: fact or artifact in spectral analysis of pulse trains. Proc Second Symp Mar Bio-Acoustics. 2(68):15-43.

Wells KD (1977) The social behavior of anuran amphibians. Anim Behav 25:66-693.

Wells KD (1988) The effect of social interactions on anuran vocal behavior. In: Fritzsch B, Ryan M, Wilczynski W, Walkowiak W, Hetherington T (Ed) The Evolution of the Amphibian Auditory System. New York, John Wiley and Sons, 433-454.

Wells KD (2007) The Ecology and Behavior of Amphibians. The University of Chicago Press, Chicago and London.

Wells KD, Schwartz JJ (2007) The Behavioral Ecology of Anuran Communication. In: Narins PM, Feng AS, Fay RR, Popper AN. (Ed.) Hearing sound communication in Amphibians. New York: Springer-Verlag.

Wilczynski W, Capranica R (1984) The auditory system of anuran amphibians. Prog Neurobiol 22:1-38. doi: 10.1016/0301-0082(84)90016-9

Wilkins MR, Seddon N, Safran RJ (2012) Evolutionary divergence in acoustic signals: Causes and consequences. Trends Ecol Evol 28(3):156-166. doi: 10.1016/j.tree.2012.10.002

Zimmermann E (1985) The vocal repertoire of the adult Senegal bushbaby (*Galago senegalensis senegalensis*). Behaviour, v. 94, p. 212-233.

Appendix 1 – Legends and Figures:

Fig. 1. Natural call with harmonics (FF: fundamental frequency and DF: dominant frequency).

Fig. 2. Acoustic stimuli, equipment disposition and dimensions of the semi-anechoic arena for female choice experiments.

Fig. 3. Acoustic stimuli (natural with harmonics: NWH, synthetic without harmonics: SWH, synthetic with dominant harmonic inverted: SDHI) and speaker disposition for the male-male interaction experiments.

Fig. 4. Choice prevalence with binomial error (A), interest timing (B), and latency (C) for female choice experiment regarding natural and synthetic (free of harmonics) stimuli. Boxplot with mean, standard error and 90 % of confidence interval.

Fig. 5. Oscillograms, rate, dominant frequency and duration of notes A, B, and C in response to three types of stimuli: i) natural with harmonics (blue), ii) without harmonics (red), and iii) dominant harmonic inverted (green).

Fig.1

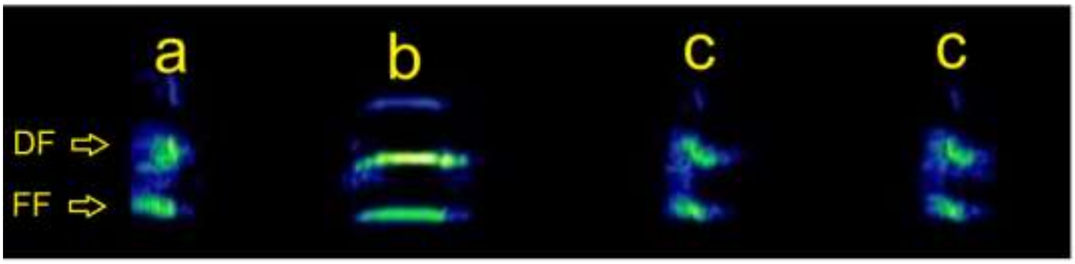


Fig.2

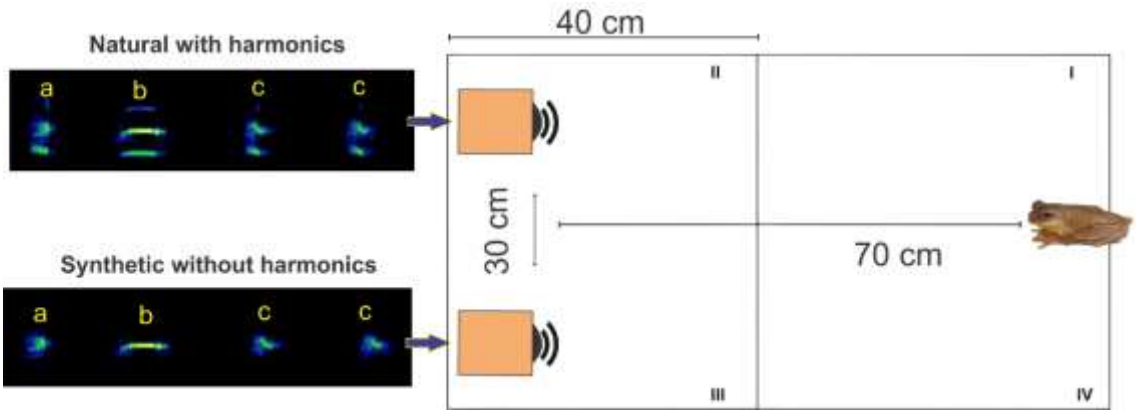


Fig.3

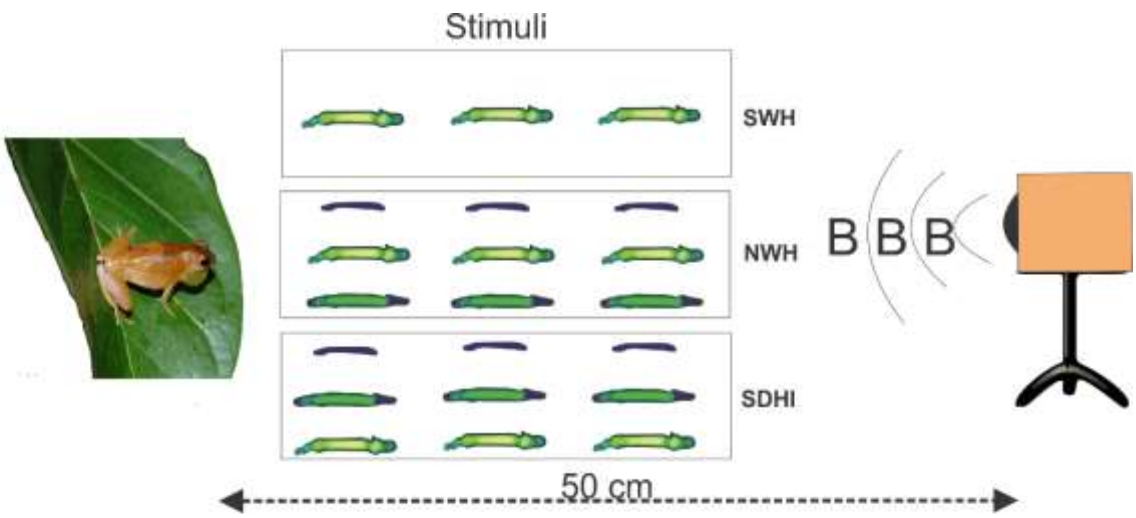


Fig.4

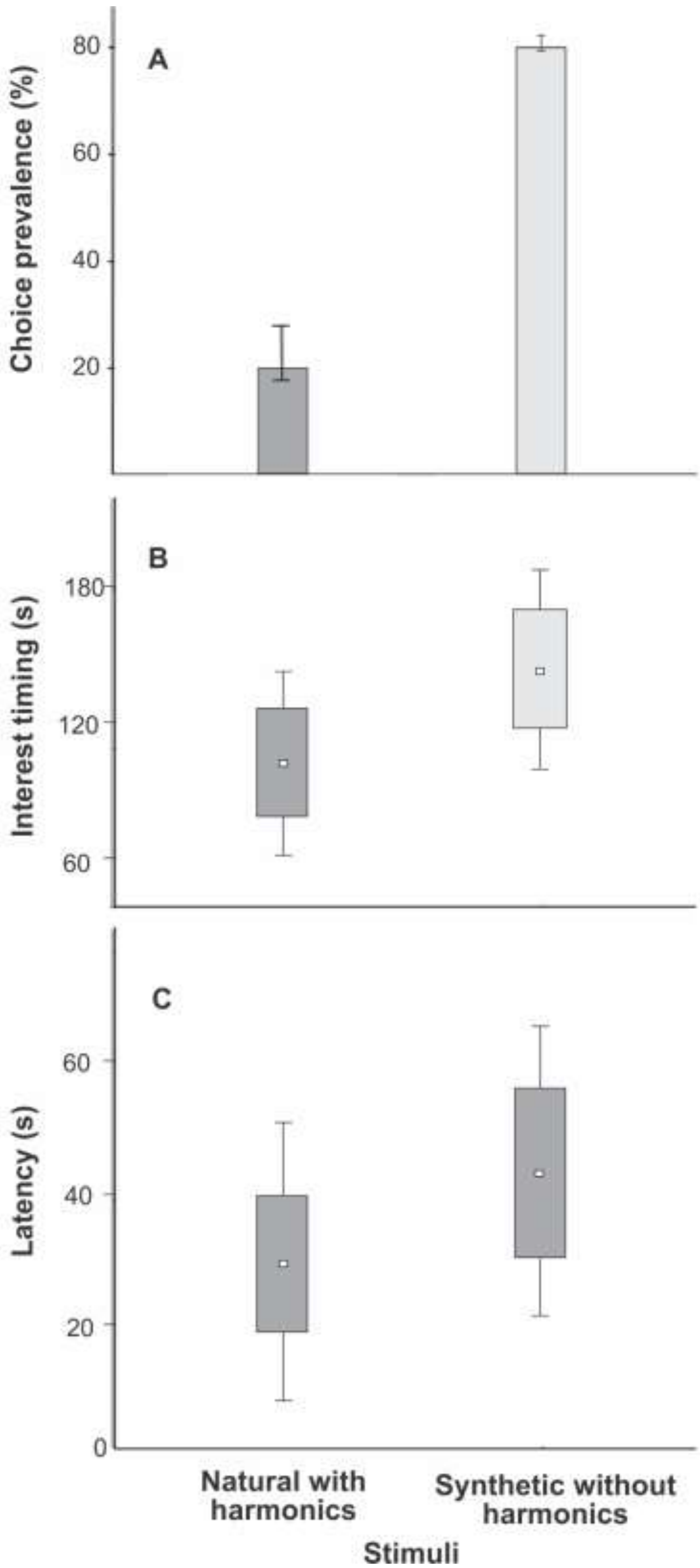
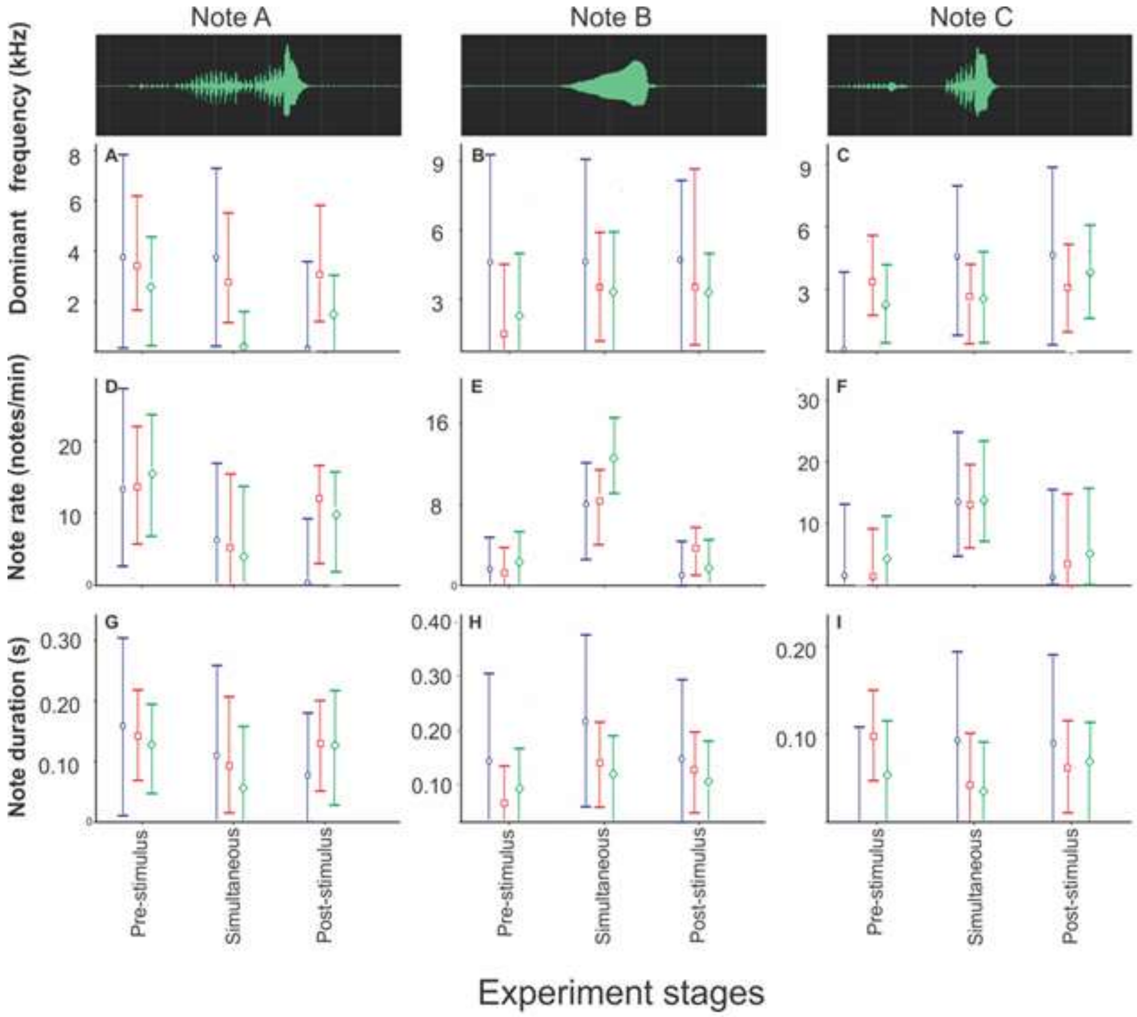

A
Choice prevalence (%)
80
60
40
20
B
Interest timing (s)
180
120
60
C
Latency (s)
60
40
20
0
Natural with harmonics
Synthetic without harmonics
Stimuli

Fig.5


Note A
Note B
Note C
Dominant frequency (kHz)
Note rate (notes/min)
Note duration (s)
A
B
C
D
E
F
G
H
I
Pre-stimulus
Simultaneous
Post-stimulus
Experiment stages

Appendix 2- Tables:

Table 1. Multiple comparison of acoustic properties among male-male interaction experimental stages. Results of a sequence of ANOVA analyses with and post-hoc test of Fischer with specific differences among experimental stages.

Stimuli	Acoustic variables	n	Degrees of freedom	F value	*P* value	Fischer test
Natural with harmonics	rate of emission of note A	12	2	-	-	-
Synthetic without harmonics	rate of emission of note A	15	2	0.756	$P = 0.701$	-
Synthetic with dominant harmonic inverted	rate of emission of note A	29	2	-	-	
Natural with harmonics	rate of emission of note B	33	2	-	-	-
Synthetic without harmonics	rate of emission of note B	16	2	-	-	-
Synthetic with dominant harmonic inverted	rate of emission of note B	29	2	-	-	-
Natural with harmonics	rate of emission of note C	32	2	-	$P = 0.015$; $P = 0.038$	Pre-stimulus ≠ Simultaneous; Simultaneous ≠ Post-stimulus
Synthetic without harmonics	rate of emission of note C	14	2	-	$P = 0.010$; P = 0.024	Pre-stimulus ≠ Simultaneous; Simultaneous ≠ Post-stimulus
Synthetic with dominant harmonic inverted	rate of emission of note C	28	2	-	$P = 0.032$; 0.024	Pre-stimulus ≠ Simultaneous; Simultaneous ≠ Post-stimulus
Natural with harmonics	Dominant frequency of note A	24	2	-	-	-
Synthetic without harmonics	Dominant frequency of note A	24	2	2.09	0.449	-
Synthetic with dominant harmonic inverted	Dominant frequency of note A	24	2	-	-	-
Natural with harmonics						
Natural with harmonics	Dominant frequency of note B	24	2	-		-
Synthetic without harmonics	Dominant frequency of note B	24	2	-		-

Synthetic with dominant harmonic inverted	Dominant frequency of note B	24	2	-	-
Natural with harmonics	Dominant frequency of note C	24	2	-	-
Synthetic without harmonics	Dominant frequency of note C	24	2	-	-
Synthetic with dominant harmonic inverted	Dominant frequency of note C	24	2	-	-
Natural with harmonics	Duration of note A	24	2	-	-
Synthetic without harmonics	Duration of note A	24	2	0.621	0.810
Synthetic with dominant harmonic inverted	Duration of note A	24	2	-	-
Natural with harmonics	Duration of note B	24	2	-	-
Synthetic without harmonics	Duration of note B	24	2	-	-
Synthetic with dominant harmonic inverted	Duration of note B	24	2	-	-
Natural with harmonics	Duration of note C	24	2	-	-
Synthetic without harmonics	Duration of note C	24	2	-	-
Synthetic with dominant harmonic inverted	Duration of note C	24	2	-	-

Figure S 1. Top view of the semi-anechoic arena used in experiments with females

Table S 1. Experiments playback field with three populations of D. minutus from the state of São Paulo, southeastern Brazil: Sorocaba; Ribeirão Grande and São Luiz do Paraitinga. Geographic coordinates, climate and type vegetation. *IBGE

	Sorocaba	Ribeirão Grande	São Luiz do Paraitinga
Geographic coordinates	23°17' S, 47°16 W	24º25' S, 48º3' W	23°20' S, 45°08' W
Climate category	Cwa (Köppen)	Cwa (Köppen)	Cwa (Köppen)
Vegetation type*	Semideciduous forest and Savannah	Dense ombrophylous forest	Dense ombrophylous forest

Table S 2. Air temperature, humidity, hour and body size (snout-vent length – SVL) for each male-male interaction experiment in each locality.

Locality	Male	Stimuli	SVL (mm)	Air temperature (°C)	Humidity (%)	Hour
Sorocaba	1	Synthetic whithout harmonics	Not measured	23.3	66	20h21
	1	Natural whith harmonics	Not measured	22.9	76	20h41
	2	Natural whith harmonics	Not measured	22.7	77	21h
	2	Synthetic whithout harmonics	Not measured	23.0	78	21h10
	3	Natural whith harmonics	Not measured	23.5	75	21h37
	3	Synthetic whithout harmonics	Not measured	23.0	70	21h29
	4	Natural whith harmonics	Not measured	22.7	73	19h52
	4	Synthetic whithout harmonics	Not measured	22.5	77	20h09
	5	Natural whith harmonics	Not measured	22.5	77	20h17
	5	Synthetic with dominant harmonic inverted	Not measured	22.6	77	20h38

	5	Synthetic with dominant harmonic inverted	Not measured	22.8	75	20h56
	6	Natural whith harmonics	Not measured	22.9	77	21h10
	6	Synthetic with dominant harmonic inverted	Not measured	23.0	76	21h24
	6	Synthetic whithout harmonics	Not measured	22.9	77	21h31
	7	Natural whith harmonics	Not measured	22.9	70	21h37
	7	Synthetic with dominant harmonic inverted	Not measured	22.8	72	22h
	7	Synthetic whithout harmonics	Not measured	22.5	73	22h14
Ribeirão Grande	8	Natural whith harmonics	Not measured	21.7	71	23h09
	8	Synthetic whithout harmonics	Not measured	21.1	73	23h19

São Luiz do Paraitinga	9	Natural whithout harmonics	Not measured	20.2	75	23h54
	9	Synthetic whitht harmonics	Not measured	20.3	75	00h06
	10	Synthetic with dominant harmonic inverted	Not measured	20.5	77	23h54
	10	Normal whith harmonics	Not measured	21.1	75	00h06
	10	Synthetic whithout harmonics	Not measured	21.5	75	00h16
	11	Synthetic with dominant harmonic inverted	18	19.6	69	22h09
	11	Natural whith harmonics	-	19.2	70	22h22
	11	Synthetic whithout harmonics	-	19.0	71	22h30
	12	Natural whith harmonics	20	18.0	79	23h15
	12	Synthetic whithout harmonics		17.3	79	23h28

	12	Synthetic with dominant harmonic inverted		17.9	80	23h45
	13	Natural whith harmonics	-	20.5	80	22h29
	13	Synthetic whithout harmonics	21	20.5	83	23h
	13	Synthetic with dominant harmonic inverted	-	20.5	83	23h07

Anexos:

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CERTIFICADO

Certificamos que a proposta intitulada **EVOLUÇÃO E FUNÇÃO DE COMPONENTES HARMôNICOS NA COMUNICAÇÃO ACÚSTICA EM ANUROS**, registrada com o nº **4395-1**, sob a responsabilidade de **Prof. Dr. Luis Felipe de Toledo Ramos Pereira e Roseli Maria Foratto**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido** aprovada pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas - CEUA/UNICAMP,** em reunião de **03 de outubro de 2016**.

Finalidade:	**( ) Ensino (x) Pesquisa Científica**
Vigência do projeto:	**15/10/2016-15/05/2017**
Vigência da autorização para manipulação animal:	**15/10/2016-15/05/2017**
Espécie / linhagem/ raça:	**Anfíbio / Dendropsophus minutus**
No. de animais:	**45**
Peso / Idade:	**01 ano / 3g**
Sexo:	**15 machos / 30 fêmeas**
Origem:	**Unidade de Conservação Estadual, Parque Estadual Intervales**

A aprovação pela CEUA/UNICAMP não dispensa autorização prévia junto ao **IBAMA**, **SISBIO** ou **CIBio**.

Campinas, 03 de outubro de 2016.

Verinaud

Profa. Dra. Liana Maria Cardoso Verinaud
Presidente

Fátima Alonso
Secretária Executiva

IMPORTANTE: Pedimos atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação de relatório no prazo estabelecido impedirá que novos protocolos sejam submetidos.

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CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **EVOLUÇÃO E FUNÇÃO DE COMPONENTES HARMÔNICOS NA COMUNICAÇÃO ACÚSTICA EM ANUROS** (protocolo CEUA/UNICAMP nº **4395-1**), de responsabilidade do **Prof. Dr. Luis Felipe de Toledo Ramos Pereira** e **Roseli Maria Foratto**, teve o título alterado para **Função dos componentes harmônicos na comunicação acústica de Dendropsophus minutus (Peters, 1872)**.

Este documento é válido apenas se apresentado junto com os certificados emitidos originalmente pela CEUA/UNICAMP, sendo emitido em 03/10/2016.

Campinas, 12 de julho de 2017.

Profa. Dra. Liana M. C. Verinaud
Presidente

Fátima Alonso
Secretária Executiva

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Campinas, 5 de fevereiro de 2018

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